

# **Optically-Active Colloidal Organic Matter and its Contribution to Variable Chromophoric DOM Signatures in Nearshore Seawaters**

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## **LONG-TERM GOAL**

My long term research goal is to ascertain the nature and magnitude of optical effects (absorbance / fluorescence / scattering) in surface seawaters associated with the production and cycling of marine colloidal organic matter. I am particularly interested in determining how these effects are driven or modulated by the productivity dynamics of phytoplankton and marine heterotrophic bacteria.

## **OBJECTIVES**

Marine chromophoric dissolved organic matter (CDOM) imparts highly variable optical signatures in surface waters over short spatial and temporal scales for reasons not yet understood. While considerable research efforts are currently underway on the specific absorption and fluorescence characteristics of the bulk CDOM, my primary objective is to follow the chromophoric signatures of different molecular weight fractions to determine if the production and removal of colloidal organic matter contributes to the high variability in bulk CDOM. We know that a significant fraction (10-40%) of non-living dissolved organic matter resides in the colloidal size fraction (1-1000 nm) (Chen and Schnitzer 1989; Benner et al. 1992; Buesseler et al. 1995), and that this fraction is very reactive to both bio-degradation (to soluble substances) (Benner et al. 1992) and aggregation (to large sinking particles) (Baskaran et al. 1992; Moran and Buesseler 1992). The challenge is to determine to what extent these dynamic, opposing processes influence the behavior of CDOM in surface seawaters, and how these biogeochemical effects interweave with photochemical degradation pathways.

## **APPROACH**

My studies combine both laboratory and field experiments which seek to determine how the optical characteristics of soluble and colloidal organic matter changes under different phytoplankton growth and light conditions. A secondary, more difficult aspect will be to tease out the heterotrophic bacterial impacts in natural population cultures.

For the laboratory component, I will be characterizing the optical character and magnitude of colloidal and soluble CDOM phases produced by diatoms in continuous cultures. Diatoms are an ideal starting point for this work because they are responsible for the highest levels of productivity in coastal waters. Diatoms will be grown under various nutrient conditions to impart different, stress-determined cellular growth rates. The continuous cultures, where cell growth is balanced by media inflow, create uniform a physiological state of the population. As a result, the CDOM signature is not the compilation of molecules produced at different cellular growth stages, as is the case in batch cultures. These cultures

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form a key component of another project but provide a unique test bed for beginning the study of the molecular size of CDOM components.

For the field component, natural population cultures from nutrient-rich upwelling waters along the California and Peruvian coastal regions will be grown in on-deck incubations over a period of several days. During my initial cruise last May, the upwelled waters were tracked over several days using drogue buoys while the phytoplankton growth was monitored. In both cases, samples are collected for size fractionation of the bulk CDOM.

I will use two methods to separate CDOM size fractions. The first is cross flow filtration (CFF) (or ultrafiltration) where seawater samples are recirculated under pressure (~ 10 psi) above a 1 Kilo Dalton membrane, a small portion of the soluble phase passing through the membrane on each cycle. After ~2 hr, the large volume (20 l) sample has been partitioned into soluble (membrane-permeable) and colloidal (membrane-retained) fractions for analysis. This method recently has become common among oceanographers studying marine organic matter.

The second approach employs Flow Field-Flow Fractionation (Flow FFF) to separate organic phases into a size continuum, from soluble through to particulate ( $> 0.45 \mu\text{m}$ ) sizes. Briefly, a flow field is applied at right angles to the channel flow within a shallow (~200  $\mu\text{m}$ ) ribbon-like chamber. Soluble fractions are driven through the membrane (1 kDa) on the accumulation wall, while colloidal components are driven to the accumulation wall. The resultant concentration gradient is opposed by diffusion (a function of colloidal size), resulting in colloids of different size being retained in different stream laminae. Unequal laminae velocities due to shear along the accumulation wall causes a well-defined separation of different colloidal size fractions according to the mean colloid proximity to the wall, which then is measured after the sample stream exits the flow chamber. In addition to yielding a soluble phase, the method provides a high resolution separation of organic matter into a continuous colloidal size spectrum.

In addition to these studies, I also will conduct photobleaching experiments to determine which CDOM components are most susceptible to photodegradation and whether this sensitivity is related to the size of matter containing the chromophore. The purpose here is to elucidate whether the harboring of fluorescent molecules within colloidal organic matrices imparts any resistance to photodestruction.

I will characterize the optical signals of the bulk and size-fractionated CDOM by acquiring 3 dimensional excitation/emission matrices (plotted as an intensity contour surface). 3D fluorescence spectroscopy has been shown to be an effective means for distinguishing between types of organic matter in seawater (Coble 1996). In addition, I will measure the absorbance matrices across the same range so that I can determine the relative fluorescence efficiencies. Combining the sensitivity of fluorescence spectroscopy with established and novel size fractionation methods will provide for the first time the opportunity to establish where CDOM components reside in the size spectrum of non-living organic matter.

## **WORK COMPLETED**

Initiation of these studies was delayed by the late arrival of the scanning spectrofluorometer, but fortunately this equipment arrived just in time for my NSF-funded cruise along the California coast in May, 1998. The 3 week cruise provided the opportunity for shipboard testing of the new equipment as well as a number of experiments. Several phytoplankton growout incubations were conducted along

with in-situ tracking of recently upwelled waters using drogue buoys. Size fractionated samples ( $<0.45\ \mu\text{m}$ ,  $< 1\ \text{kDa}$ ,  $> 1\ \text{kDa}$ ) were collected from these incubation experiments.

In addition to these experiments, several probe experiments also were conducted to investigate the comparative hydrophobicity of the colloidal organic phases present in these incubations. This information is relevant to the study of CDOM because it helps predict what type of molecules can become associated with the bulk colloidal organic phase. (An alternate view is that CDOM molecules themselves are very large— ie., colloidal — molecules.) Fluorescein, Lucifer Yellow, Tetramethyl Rhodamine B, and Bodipy COOH were spiked at trace concentrations into natural population cultures and the size fractionation of these probes followed over several days.

Sample analysis from this cruise have proceeded well. The preliminary fluorescence analyses of all samples now has been completed, though I intend to reanalyze many of these samples for other optical properties (fluorescence polarization, temperature dependence of fluorescence, absorption matrices).

I also have started a preliminary study of how additions of high concentrations of EDTA affect the natural fluorescence signatures. Published work earlier this year suggests that colloidal organic phases in seawater have the characteristics of macrogels; tangled networks of natural polymers held together by ion bridging. One aspect of such gels is that they should disintegrate upon the addition of EDTA due to the lowering of  $\text{Mg}^{2+}$  and  $\text{Ca}^{2+}$  free ion activities (the primary bridging ions). If chromophores are associated with these gels, this release would change their chemical microenvironment which, in turn, could significantly affect their fluorescence characteristics. The aim of these experiments is to examine how microbial degradation of the colloidal matrix might affect CDOM fluorescence.

Development of continuous cultures has taken considerable effort over the past months but the apparatus has now been fabricated and the methodologies established. The challenge has been to reliably attain steady-state growth conditions; a necessary goal to ensure a stable physiological state of the cells.

The key individuals assisting me with this work are Bettina Sohst and Peggy Hughes, both research specialists here in the Institute of Marine Sciences, UCSC.

## RESULTS

Unusually mild coastal upwelling conditions were encountered during the research cruise last May as a result of the intense El Nino event. Surface macronutrient concentrations in these weak upwelling centers were only mildly elevated (e.g.  $\sim 4\ \mu\text{M}$  nitrate) compared to previous years ( $\sim 20\text{-}25\ \mu\text{M}$ ). Phytoplankton growth in the cultures therefore did not yield as high a biomass as normal. Nonetheless, significant changes in the fluorescent component of CDOM were seen in relation to phytoplankton growth.

1. The fluorescent component of CDOM increased in cultures and in the tracked upwelled surface waters. However, these increases were not uniform at all excitation/emission wavelengths but were seen as a deformation of the fluorescence surface. For example, in one bioassay, a nitrate drawdown of  $1.5\ \mu\text{M}$  led to a 20% increase in “humic-type” fluorescence (ex 320; em 400) but a 400% increase in “protein-type” fluorescence (ex 275; em 340).

2. The percentage of “humic-type” material (ex 320; em 400) that was colloidal (> 1 kDa) changed during the course of the bioassay. In the recently upwelled waters, values were ~ 2% colloidal but then increased over the first few days to as much as 50% and then dropped back down after the macronutrients were drawn to zero. The cause of the decrease is not known but it would have been due to colloid aggregation or bacterial degradation (photolysis would not be significant because UV light would have been absorbed by the 0.5 in thick plexiglas walls of the incubators).
3. Not surprisingly, the “protein-type” fluorescence signature (ex 274; em 340) was greatly intensified in the cross-flow filtration retentates (i.e., > 1 kDa), demonstrating that much of this material is colloidal in nature. However, these preliminary results show greater increases than can be explained by simple preconcentration of the colloidal phase (by CFF); i.e., new “protein-type” fluorescence appeared in the retentates and permeates (< 1 kDa). One possible explanation is that a fluorescence contaminant was introduced by the CFF system; a possibility that can be assessed when the Flow Field-Flow Fractionation system comes on-line. However, another more exciting possibility is described below
4. Based on preliminary results, addition of EDTA to conventionally filtered (< 0.45  $\mu$ m) seawater dramatically alters the fluorescence surface of the excitation/emission matrix. The largest effect is an increase in “protein-type” fluorescence. These results, consistent among several samples from different regions, suggest that colloidal gel formation encases proteins into non-polar (or hydrophobic) chemical microenvironments that reduce the fluorescence efficiency. Partial breakup of these gels should release the proteins to a more hydrophilic microenvironment, perhaps increasing their fluorescence. Shear forces during CFF may cause a similar effect if the gels are susceptible to physical breakup, which might explain the disproportionately large increase in “protein-type” fluorescence noted above. If so, then this effect should not be seen with Flow Field-Flow Fractionation, where shear forces are much less.
5. The fluorescent probes, having different degrees of hydrophobicity, showed different affinities for the colloidal organic phase in growing phytoplankton cultures. My preliminary results indicate that this affinity increased with hydrophobicity of the probe, although differences were small. The probe experiments will have to be expanded to include molecules having a greater range of water solubility before firm conclusions can be made.

## IMPACT/APPLICATION

These preliminary results support the view that a significant fraction of the CDOM fluorescence component is associated with the marine colloidal phase. This colloidal fraction of CDOM can change rapidly in conjunction with phytoplankton growth, ranging from a few percent to roughly half the bulk CDOM and back again within several days. Moreover, it appears likely that at least some fraction of these fluorescent molecules are low molecular weight substances which partition into colloidal sized assemblages of molecules (macrogels). Combined, these findings strongly suggest that colloid aggregation could rapidly transfer “dissolved” chromophoric matter into sinking particles, removing them from remotely-sensed surface waters on the time scale of hours. In addition, microbial degradation of the colloidal matrices may alter the fluorescent signature of CDOM by changing the chromophores chemical microenvironment from more hydrophobic to more hydrophilic. These findings provide insights to the underlying biogeochemical mechanisms affecting the magnitude and character of CDOM in seawater and complement those studies examining direct photochemical effects.

## **TRANSITIONS**

At this early juncture of the project there have been no linkages formed with other colleagues.

## **RELATED PROJECTS**

The laboratory continuous culture experiments are joint with my ECOHAB project with Dave Garrison (UCSC/NSF) and Ron Tjeerdema (UCSC). The primary goal of that project is to investigate factors contributing to the production of the toxin domoic acid by *Pseudo-Nitzschia* sp.

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- Wells, M. L. 1998. Marine colloids: a neglected dimension. *News and Views, Nature* 391:530-531.